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Evaluation of Plasma Zinc and Copper in Patient with Chronic Renal Failure in Khartoum State

A Dissertation Submitted in Partial Fulfillment of the Requirement of Master (M.Sc.) Degree in Medical Laboratory Science (Clinical Chemistry)

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الآية

بسم الله الرحمن الرحيم

قال تعالي :

" وفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٍ " (١)

" وَقُل رَبِّ زِدْنِي عِلْمَاً "(٢)

صدق الله العظيم

(١)سورة يوسف : الآية ٧٦

(٢) سورة طه : الآية ١١٤

Dedication

To the one who taught me how to be available member in community?

Dear father

To the depth of be longings and rhythm of sympathy

Dear mother

To crown of pleasure and secret of existence

My wife and kids

I would like to thank my wife Aesha for standing beside me throughout my study. This research is dedicated to my kids Sheema, Abdalbagi, Arwa and Mutaz

Brothers

To partners of educational years

Friend and dear colleagues and special for golden mansion sons

And special dedication for every one that helped me to reach this place

Acknowledgment

All thanks to Allah from the start to the end…..

And pray for Prophet Mohammed peace be upon him

I would like to acknowledge the contribution of my

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Dr/ Abdelwahab Abdien Saeed

Who guide me throughout my way and helped me to make this research as accurate and useful as possible.

And I'm grateful to my friends and all those who contributed their time and helped me.

My thanks also extend to my college and my teacher

Abstract

Background: The kidney are complex vital organs, and have many function. The main function it‘s removal of toxic and excess substances from the plasma, if there is any defect in the kidney like renal failure can disrupt this function. The aim of this study was to determine the level of Zinc and Copper in Sudanese patient with chronic renal failure.

Material and Methods: This study was designed as case control, which includes 100 blood samples, a 60 from these sample were collected from patient with chronic renal failure and 40 samples were collected from health individual as control group and the sample is collected by using sterile disposable syringes and separated by centrifuge. Carried out in Ribat University Hospital in Khartoum state, during period from March to June 2018. And the plasma levels of zinc and copper determined by the use of atomic absorption spectrophotometer (OPERATOR’S MANUAL January 2003 VER 3.94 C), and the obtained results were analyzed by SPSS.

Result: The result of this study showed that there was significant decrease ($p<0.05$) in the plasma levels of zinc and copper in patient with chronic renal failure compared to the control subjects. The mean of plasma Zn was 0.3mg/l in test group and 0.7mg/l in control group with p.value of 0.002 and the mean of plasma Copper was 0.5mg/l in test group and 0.7mg/l in control group with p.value of 0.019. Also the study showed the gender and age of the patient, also the duration of the disease have no effect on the plasma level of zinc and copper ($p>0.05$).

Conclusion: The study conclude that the plasma level of zinc and copper are low in patient with chronic renal failure. And the gender and age of the patient also the duration of disease have no significant effect on the plasma level of zinc and copper.

المستخلص

خلفية: الكلية عضوء حيوي معقّدَ، الوظيفة الرئيسية للكلية هي إزالةُ الموادِ الغير مرغوبةِ مِنْ البلازما ، في حالة المرض الكلوي ، الكلية قَدْ تخسر هذه الوظيفةِ. هدفة هذه الدراسة الي تحديد مستوى معدني (النحاس والزنك) في مرضى الفشل الكلوي بالسودان.

الادوات والطرق: اجريت هذه الدراسة (دراسة الحالة والحالة الضابطة) في مستشفي الرباط الجامعي بالخرطوم ، تم الفحص علي 60 مريض يعانون من الفشل الكلوي , تتراوح اعمارهم بين 17 الي 90 سنة ، و 40 شخص غير مريض كمجموعة ضابطة وقد تم جمع العينات باستخدام حقن معقمة وفصلها بواسطة استخدام جهاز الطرد المكزي ، في الفترة مابين شهر مارس الي يونيو 2018 ، وقد تم قياس مستوى المعادن بواسطة استخدام جهاز الامتصاص الذري , والنتائج التي تم الحصول عليها حللت باستخدام برنامج اس بي اس اس.

النتائج: فقد وضح من هذه الدراسة انخفاض معنوي ($p<0.05$) في مستوى معدني النحاس والزنك في مرضي الفشل الكلوي الحاد مقارنة مع المجموعة الضابطة. متوسط مستوى الزنك لدى المرضى 0.3mg/l وفي العينة الضابطة 0.7mg/l والقيمة الاحتمالية كانت 0.002 كما ان متوسط مستوى النحاس لدى المرضى 0.5mg/l وفي العينة الضابطة كانت 0.7mg/l و القيمة الاحتمالية 0.019. كما اوضحت هذة الدراسة ايضا عدم تأثر في مستوى معدني النحاس والزنك بالعمر وجنس المريض، وايضا لم يتاثرا بالفترة الزمنية للمرض ($p>0.05$).

الخاتمة: و قد نتج من هذة الدراسة ان مرض الفشل الكلوي الحاد ياثرعلي مستوى معدني النحاس والزنك ، مستوى الزنك والنحاس ينقصان في حالة الفشل الكلوي الحاد ولكن عمر وجنس المريض والمدة الزمنية للمرض ليس لهما تأثير هام على مستوى الزنك والنحاس في البلازما.

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List of Abbreviations

Abbreviation	Meaning
BUN	Blood Urea Nitrogen
CKD	Chronic Kidney Disease
CAPD	Continuous Ambulatory Peritoneal Dialysis
Cu	Copper
ESRD	End Stage Renal Disease
GFR	Glomerular Filtration Rate
HLA	Human Leukocyte Antigen
RTA	Renal Tubular Acidosis
RBCs	Red Blood Cells
SLE	Systemic Lupus Erythematosus
USRDS	United States Renal Data System
Zn	Zinc
SPSS	Statistical Package for Social Sciences

Chapter One

Introduction
Rationale
Objectives

1.1 Introduction

The kidneys are vital organs that perform a variety of important functions. The most prominent functions are removal of unwanted substances from plasma (both waste and surplus), homeostasis (maintenance of equilibrium) of the body's water, electrolyte and acid base status, and participation in hormonal regulation.[1]

The renal failure may be acute or chronic, acute renal failure is a sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys, defined as occurring when the GFR is reduced to less than 10 mL/minute. This syndrome is subdivided into three types, depending on the location of the precipitating defect. And chronic kidney disease (CKD) is a clinical syndrome that occurs when there is a gradual decline in renal function over time. According to the 2007 U.S. Renal Data System (USRDS) Annual Data Report, one in nine U.S. adults has CKD and 20 million more are at risk. Early detection and treatment are needed to prevent progression to ESRD and complications such as coronary vascular disease. The National Kidney Foundation has formulated guidelines for earlier diagnosis, treatment, and prevention of further disease progression. GFR and evidence of kidney damage based on measurement of proteinuria or other markers form the basis of the classifications. The conditions that can precipitate acute renal failure also may lead to chronic renal failure.[2]

The essential trace elements are usually associated with an enzyme (metalloenzyme) or another protein (metalloprotein) as an essential component or cofactor. Deficiencies typically impair one or more biochemical functions and excess concentrations are associated with at least some degree of toxicity. Although trace elements, such as iron, copper, and zinc, are found in mg/L concentrations. An element is

considered essential if a deficiency impairs a biochemical or functional process and replacement of the element corrects this impairment. Decreased intake, impaired absorption, increased excretion, and genetic abnormalities are examples of conditions that could result in deficiency of trace elements. The cells of the proximal renal tubule have an important role in the homeostasis of essential metals, and the kidney is a target site for metal toxicity. For this reason, and because of their close chemical similarity and extensive biological interaction, they are often considered together.[3]

1.2 Rationale

The kidneys are vital organs that perform a variety of important functions and any disease of the kidney lead to defect in the renal function like regulation of the concentration of some substances. The essential trace metals are involved in a number of metabolic activities, including neuroconduction, transport, excretory processes and serving as cofactors for enzymes. The cells of the proximal renal tubule have an important role in the homeostasis of essential metals, and the kidney is a target site for metal toxicity. Zinc and copper are two of the most intensively investigated and metabolically important trace metal nutrients. For this reason, and because of their close chemical similarity and extensive biological interaction, they are often considered together.

This study will try to detect if there is any change in the concentration of zinc and copper in patient with renal failure.

1.3 Objective

1.3.1 General objective

To evaluate the trace elements (Zinc and Copper) in chronic renal failure patients and control group.

1.3.2 Specific objectives

1. To measure plasma zinc and copper levels in healthy individuals as control group and in patients with chronic renal failure as case group.
2. To compare the trace elements (Zinc and Copper) in chronic renal failure patients according to gender.
3. To compare the trace elements (Zinc and Copper) in chronic renal failure patients according to age.
4. To determine the effect of chronic renal failure duration on trace elements (Zinc and Copper).

Chapter Two

Literature Review

2 Literature review

2.1 Kidneys

The kidneys are vital organs that perform a variety of important functions. The most prominent functions are removal of unwanted substances from plasma (both waste and surplus), homeostasis (maintenance of equilibrium) of the body's water, electrolyte and acid-base status, and participation in hormonal regulation. In the clinical laboratory, kidney function tests are used in assessment of renal disease, water balance, and acid-base disorders and in situations of trauma, head injury, surgery, and infectious disease.(4)

2.1.1 Renal anatomy

The kidneys are paired, bean-shaped organs located retroperitoneally on either side of the spinal column. Macroscopically, a fibrous capsule of connective tissue encloses each kidney. When dissected longitudinally, two regions can be clearly discerned an outer region called the cortex and an inner region called the medulla. The pelvis can also be seen. It is a basin like cavity at the upper end of the ureter into which newly formed urine passes. The bilateral ureters are thick-walled canals, connecting the kidneys to the urinary bladder. Urine is temporarily stored in the bladder until voided from the body by way of the urethra. Nephron in the functional units of the kidney that can only be seen microscopically. Each kidney contains approximately 1 million nephrons. Each nephron is a complex apparatus comprised of five basic parts.

1. Glomerulus
2. Proximal convoluted tubule
3. loop of Henle
4. Distal convoluted tubule
5. Collecting duct.(5)

2.1.2 Renal physiology

There are three basic renal processes:

1. Glomerular filtration
2. Tubular reabsorption
3. Tubular secretion

2.1.3 Pathophysiology

2.1.3.1 Glomerular Diseases

Disorders or diseases that directly damage the renal glomeruli may, at least initially, exhibit normal tubular function. With time, however, disease progression involves the renal tubules, as well. The following syndromes have discrete symptoms that are recognizable by their patterns of clinical laboratory findings.[6]

a. Acute Glomerulonephritis

Pathologic lesions in acute glomerulonephritis primarily involve the glomerulus. Histologic examination shows large, inflamed glomeruli with a decreased capillary lumen. Abnormal laboratory findings usually include rapid onset of hematuria and proteinuria (usually albumin, and generally <3 g/day). The rapid development of a decreased GFR, anemia, elevated blood urea nitrogen (BUN) and serum creatinine, oliguria, sodium and water retention (with consequent hypertension and some localized edema), and, sometimes, congestive heart failure is typical. Numerous hyaline and granular casts are generally seen on UA. The actual RBC casts are regarded as highly suggestive of this syndrome. Acute glomerulonephritis is often related to recent infection by group A β-hemolytic streptococci. It is theorized that circulating immune complexes trigger a strong inflammatory response in the glomerular basement membrane, resulting in a direct injury to the glomerulus itself. Other possible causes include drug-related exposures, acute kidney infections due to other bacterial (and, possibly, viral) agents, and other

systemic immune complex diseases, such as systemic lupus erythematosus (SLE) and bacterial endocarditis.[7]

b. Chronic Glomerulonephritis

Lengthy glomerular inflammation may lead to glomerular scarring and the eventual loss of functioning nephrons. This process often goes undetected for lengthy periods because only minor decreases in renal function occur at first and only slight proteinuria and hematuria are observed. Gradual development of uremia (or azotemia, excess nitrogen compounds in the blood) may be the first sign of this process. [8]

c. Nephrotic syndrome

Nephrotic syndrome can be caused by several different diseases that result in injury and increased permeability of the glomerular basement membrane. This defect almost always yields several abnormal findings, such massive proteinuria (>3.5 g/day) and resultant hypoalbuminemia. The subsequent decreased plasma oncotic pressure causes a generalized edema as a result of the movement of body fluids out of vascular and into interstitial spaces. Other hallmarks of this syndrome are hyperlipidemia and lipiduria. Lipiduria takes the form of oval fat bodies in the urine. These bodies are degenerated renal tubular cells containing reabsorbed lipoproteins. Primary causes are associated directly with glomerular disease states. [9]

2.1.3.2Tubular Diseases

Tubular defects occur to a certain extent in the progression of all renal diseases as the GFR falls. In some instances, however, this aspect of the overall dysfunction becomes predominant. The result is decreased excretion/ reabsorption of certain substances or reduced urinary concentrating capability. Clinically, the most important defect is renal tubular acidosis (RTA), the primary tubular disorder affecting acid-base

balance. This disease can be classified into two types, depending on the nature of the tubular defect:

Distal RTA, in which the renal tubules are unable to keep up the vital pH gradient between the blood and tubular fluid.

Proximal RTA, in which there is decreased bicarbonate reabsorption, resulting in hyperchloremic acidosis. In general, reduced reabsorption in the proximal tubule is manifested by findings of abnormally low serum values for phosphorus and uric acid and by glucose and amino acids in the urine. In addition, there may be some proteinuria (usually <2 g/day).

Acute inflammation of the tubules and surrounding interstitium also may occur as a result of analgesic drug or radiation toxicity, methicillin hypersensitivity reactions, renal transplant rejection, and viral-fungal-bacterial infections. Characteristic clinical findings in these cases are decreases in GFR, urinary concentrating ability, and metabolic acid excretion; leukocyte casts in the urine; and inappropriate control of sodium balance.[10]

2.1.3.3 Urinary tract infection/obstruction

Infection

The site of infection may be either in the kidneys (pyelonephritis) or in the urinary bladder (cystitis). In general, a microbiologic colony count of more than 105 colonies/mL is considered diagnostic for infection in either locale. Bacteriuria (as evidenced by positive nitrite dipstick findings for some organisms), hematuria, and pyuria (leukocytes in the urine, as shown by positive leukocyte esterase dipstick) are all frequently encountered abnormal laboratory results in these cases. In particular, WBC (leukocyte) casts in the urine is considered diagnostic for pyelonephritis.[11]

Obstruction

Renal obstructions can cause disease in one of two ways. They may either gradually raise the intratubular pressure until nephrons necrose and chronic renal failure ensues, or they may predispose the urinary tract to repeated infections.

Obstructions may be located in the upper or lower urinary tract. Blockages in the upper tract are characterized by a constricting lesion below a dilated collecting duct. Obstructions of the lower tract are evidenced by the residual urine in the bladder after cessation of micturition (urination); symptoms include slowness of voiding, both initially and throughout urination. Causes of obstructions can include neoplasms (e.g., prostate/bladder carcinoma or lymph node tumors constricting ureters), acquired diseases (e.g., urethral strictures or renal calculi), and congenital deformities of the lower urinary tract. The clinical symptoms of advancing obstructive disease include decreased urinary concentrating capability, diminished metabolic acid excretion, decreased GFR, and reduced renal blood flow. Laboratory tests useful in determining the nature of the blockage are urinalysis, urine culture, BUN, serum creatinine, and CBC. Final diagnosis is usually made by radiologic imaging techniques.[12]

2.1.3.4 Renal calculi

Renal calculi, or kidney stones, are formed by the combination of various crystallized substances, which are listed in Table 26-2. Of these, calcium oxalate stones are by far the most commonly encountered, particularly in the tropics and subtropics.

It is currently believed that recurrence of calculi in susceptible individuals is a result of several causes but mainly a reduced urine flow rate (related to a decreased fluid intake) and saturation of the urine with large amounts of essentially insoluble substances. Chemical analysis of stones is

important in determining the cause of the condition. Specialized x-ray diffraction and infrared spectroscopy techniques are widely used for this purpose. Clinical symptoms are, of course, similar to those encountered in other obstructive processes: hematuria, urinary tract infections, and characteristic abdominal pain. [13]

2.1.3.5 Renal Failure

2.1.3.5.1 Acute renal failure

Acute renal failure is a sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys, defined as occurring when the GFR is reduced to less than 10 mL/minute. This syndrome is subdivided into three types, depending on the location of the precipitating defect.

Prerenal failure: The defect lies in the blood supply before it reaches the kidney. Causes can include cardiovascular system failure and consequent hypovolemia.

Primary renal failure: The defect involves the kidney. The most common cause is acute tubular necrosis; other causes include vascular obstructions/inflammations and glomerulonephritis.

Postrenal failure: The defect lies in the urinary tract after it exits the kidney. Generally, acute renal failure occurs as a consequence of lower urinary tract obstruction or rupture of the urinary bladder.

Toxic insults to the kidney that are severe enough to initiate acute renal failure include hemolytic transfusion reactions, myoglobinuria due to rhabdomyolysis, heavy metal/solvent poisonings, antifreeze ingestion, and analgesic and aminoglycoside toxicities. These conditions directly damage the renal tubules. Hypoxic insults include conditions that severely compromise renal blood flow, such as septic/hemorrhagic shock, burns, and cardiac failure. The most commonly observed symptoms of acute renal failure are oliguria and anuria (<400 mL/day). The

diminished ability to excrete electrolytes and water results in a significant increase in extracellular fluid volume, leading to peripheral edema, hypertension, and congestive heart failure. Most prominent, however, is the onset of the uremic syndrome or ESRD, in which increased BUN and serum creatinine values are observed along with the preceding symptoms. The outcome of this disease is either recovery or, in the case of irreversible renal damage, progression to chronic renal failure. (14)

2.1.3.5.2 Chronic renal failure (Chronic kidney disease)

Chronic kidney disease (CKD) is a clinical syndrome that occurs when there is a gradual decline in renal function over time. According to the 2007 U.S. Renal Data System (USRDS) Annual Data Report, one in nine U.S. adults has CKD and 20 million more are at risk. Early detection and treatment are needed to prevent progression to ESRD and complications such as coronary vascular disease. The National Kidney Foundation has formulated guidelines for earlier diagnosis, treatment, and prevention of further disease progression. GFR and evidence of kidney damage based on measurement of proteinuria or other markers form the basis of the classifications. The conditions that can precipitate acute renal failure also may lead to chronic renal failure.(15)

2.1.4 Therapy of acute renal failure

Dialysis

In patients with acute renal failure, uremic symptoms, uncontrolled hyperkalemia, and acidosis have traditionally been indications that the kidneys are unable to excrete the body's waste products and a substitute method in the form of dialysis was necessary. Dialysis is often instituted before this stage, however. Several forms of dialysis are available; however, they all use a semipermeable membrane surrounded by a dialysate bath. (2)

In traditional hemodialysis (removal of waste from blood), the membrane is synthetic and outside the body. Arterial blood and dialysate are pumped at high rates (150–250 mL/min and 500 mL/min, respectively) in opposite directions. The blood is returned to the venous circulation and the dialysate discarded. The diffusion of low-molecular-weight solutes (<500 Da) into the dialysate is favored by this process, but mid-molecular weight solutes (500–2000 Da) are inadequately cleared. Creatinine clearance is about 150–160 mL/min.

In peritoneal dialysis, the peritoneal wall acts as the dialysate membrane and gravity is used to introduce and remove the dialysate. Two variations of this form are available, continuous ambulatory peritoneal dialysis (CAPD) and continuous cycling peritoneal dialysis; however, the process is continuous in both, being performed 24 hours a day, 7 days a week. This method is not as rigorous as the traditional method. Small solutes (e.g., potassium) have significantly lower clearance rates compared with the traditional method, but more large solutes are cleared and steady-state levels of blood analytes are maintained.[16]

Continuous arteriovenous hemofiltration (ultrafiltration of blood), continuous venovenous hemofiltration, continuous arteriovenous hemodialysis, and continuous venovenous hemodialysis together make up the slow continuous renal replacement therapies developed to treat acute renal failure in critically ill patients in intensive care settings. In these methods, the semipermeable membrane is again outside the body. Solutes up to 5000 Da (the pore size of the membranes) and water are slowly (10 mL/min) and continuously filtered from the blood in the first two methods, causing minimal changes in plasma osmolality. Volume loss can be replaced in the form of parenteral nutrition and intravenous medications. The final two methods are similar to the filtration methods, but a continuous trickle of dialysis fluid is pumped past the dialysis

membrane, resulting in continuous diffusion and a doubling of the urea clearance. [17]

2.1.5 Therapy of end-stage renal disease

For patients with irreversible renal failure, dialysis and transplantation are the only two therapeutic options. Initiation of either treatment occurs when the GFR falls to 5 mL/min (10–15 mL/min in patients with diabetic nephropathy).[18]

2.1.5.1 Dialysis

Traditional hemodialysis or its more recent, highefficiency form, as well as peritoneal dialysis are the available methods. The clinical laboratory used in conjunction with a hemodialysis facility must be able to adequately monitor procedural efficiency in a wide variety of areas. Renal dialysis has basic goals, and specific laboratory tests should be performed to evaluate the achievement of each goal.[19]

2.1.5.2 Transplantation

The most efficient hemodialysis techniques provide only 10%–12% of the small solute removal of two normal kidneys and considerably less removal of larger solutes. Even patients who are well dialyzed have physical disabilities and decreased quality of life. Kidney transplantation offers the greatest chance for full return to a healthy, productive life. However, this option is limited by the significant shortage of donor organs. For ESRD patients, waiting for an organ donation can vary from several months to several years. [20]

Renal transplantation is from a compatible donor to a recipient suffering from irreversible renal failure. The organ can be from a cadaver or a live individual (80% and 20%, respectively, of all kidney transplants in the United States). For this procedure to be successful, the body's immune response to the transplanted organ must be suppressed. Therefore, the donor and recipient are carefully screened for ABO blood group, human

leukocyte antigen (HLA) compatibility, and preformed HLA antibodies. The HLA system is the major inhibitor to transplantation. [20]

Although kidney transplants have the capacity to function for decades, the mean half-life of a cadaveric transplant is approximately 7 years. The mortality rate is not significantly different from hemodialysis. Three-year graft survival figures vary from 65% to 85%, with live grafts doing better. It has been reported that there is no difference in patient survival among hemodialysis, CAPD, and cadaveric kidney transplantation. Live related-donor transplantation is associated with a better patient survival than other ESRD therapeutic options.[20]

2.2 Copper

Copper (Cu) is a relatively soft yet tough metal with excellent electrical and heat conducting properties. Copper is widely distributed in nature both in its elemental form and in compounds. Copper forms alloys with zinc (brass), tin (bronze), and nickel (cupronickel, widely used in coins).

2.2.1 Health effects

The copper content in the normal human adult is 50–120 mg. Copper is distributed through the body with the highest concentrations found in liver, brain, heart, and kidneys. Hepatic copper accounts for about 10% of the total copper in the body. Copper is also found in cornea, spleen, intestine, and lung.

Copper is a component of several metalloenzymes, including ceruloplasmin, cytochrome C oxidase, superoxide dismutase, tyrosinase, metallothionein, dopamine-β- hydroxylase, lysyl oxidase, clotting factor V, and an unknown enzyme that cross-links keratin in hair.

Ceruloplasmin is the best known yet the least understood copper protein. It is and α_2-globulin, and each 132,000-molecular-weight molecule contains six atoms of copper. Ceruloplasmin levels are influenced by hormones. [21]

2.2.2 Absorption, transport and excretion

An average day's diet may contain 10 mg or more of copper. The amount of copper absorbed from the intestine is 50%–80% of ingested copper. About half of dietary copper is excreted in feces. The exact mechanisms by which copper is absorbed and transported by the intestine are unknown. Copper absorption is impaired in severe diffuse diseases of small bowel, lymph sarcoma, and scleroderma. Copper losses in the urine and sweat are approximately 3% of dietary intake. Menstrual losses of copper are minor. [22]

2.2.3 Deficiency

Copper deficiency is observed in premature infants. Copper deficiency is related to malnutrition, malabsorption, chronic diarrhea, hyperalimentation, and prolonged feeding with low-copper, total-milk diets. Signs of copper deficiency include (1) neutropenia and hypochromic anemia in the early stages, (2) osteoporosis and various bone and joint abnormalities that reflect deficient copper-dependent cross linking of bone collagen and connective tissue, (3) decreased pigmentation of the skin and general pallor, and (4), in the later stages, possible neurologic abnormalities (hypotonia, apnea, psychomotor retardation). [23]

Subclinical copper depletion contributes to an increased risk of coronary heart disease. An extreme form of copper deficiency is seen in Menkes disease. This invariably fatal, progressive brain disease is characterized by peculiar hair, called kinky or steely, and retardation of growth. Clinical forms include progressive mental deterioration, coarse feces, disturbance of muscle tone, seizures, and episodes of severe hypothermia. Symptoms of Menkes disease usually appear at the age of 3 months and death usually occurs in 5-year olds. [23]

2.2.4 Toxicity

Wilson's disease is a genetically determined copper accumulation disease that usually presents between the ages of 6 and 40 years. Its manifestations include neurologic disorders, liver dysfunction, and Kayser-Fleischer rings (green-brown discoloration) in the cornea caused by copper deposition. Early diagnosis of Wilson's disease is important because complications can be effectively prevented and in some cases the disease can be halted with use of zinc acetate or chelation therapy. [24]

2.2.5 Reference intervals for copper

Copper in serum: 700–1500 μg/L12; mean levels for copper serum in women and children are slightly higher; values for blacks are 8–12% higher.

Copper in serum (pregnancy at term): 118–320 μg/L

Copper in urine: 15 – 60 μg/24 hours

Or 3 – 35 μg/24 hours or 2 – 80 μg/L

Copper in RBCs: 90–150 μg/L

2.3 Zinc

Zinc (Zn) is a bluish white, lustrous metal. Zinc is stable in dry air and becomes covered with a white coating when exposed to moisture. Zinc is the fourth most used metal (after iron, aluminum, and copper). Zinc and its compounds are used in a production of alloys, especially brass (with copper), in galvanizing steel, in die casting, in paints, in skin lotions, in treatment of Wilson's disease, and in many over-the-counter (OTC) medications. [25]

2.3.1 Health effects

Zinc is second only to iron in importance as an essential trace element. The main biochemical role of zinc is its influence on the activity of more than 300 enzymes (from the classes of oxireductases, transferases, hydrolases, lyases, isomerases, and ligases). Zinc can be essential for the

structure, regulation, and catalytic action of an enzyme. Zinc occurs in enzymes that realize the synthesis and metabolism of DNA and RNA. Zinc influences the synthesis and metabolism of proteins, participates in glycolysis and cholesterol metabolism, maintains membrane structures, effects functions of insulin, and affects growth factor.

Chronic oral zinc supplementation interferes with copper absorption and may cause copper deficiency. This ability to interfere with copper absorption is also the basis for using zinc to treat Wilson's disease. Copper status should be monitored in patients on long-term zinc therapy.[26]

2.3.2 Absorption, transport and excretion

The body content in a normal individual is about 2.5 g zinc, which is mainly in muscles (60%) and skeleton (30%). The remaining 10% is distributed in all tissues with highest concentrations in eyes, prostate, and hair. All tissue levels depend on age. Zinc absorption mainly occurs in the small intestine and especially in the jejunum. In blood, the absorbed zinc is distributed between RBCs (80%), plasma (17%), and white blood cells (3%). Different factors modify the absorption of zinc. The factors increasing zinc absorption include: presence of animal proteins and amino acids in a meal, intake of calcium, and unsaturated fatty acids. The factors decreasing zinc absorption include intake of iron, taking zinc on empty stomach, presence of copper at high levels, and age. In normal dietary circumstances, about 90% of zinc is excreted in feces. [27]

2.3.3 Deficiency

Nutritional zinc deficiency is widespread all over the world. Zinc deficiency causes growth retardation, slows skeletal maturation, causes testicular atrophy, and reduces taste perception. Old age, pregnancy, lactation, and alcoholism are also associated with poor zinc nutrition. Infants with acrodermatatisenteropathica (zinc malabsorption) usually

first develop characteristic facial and diaper rash. Untreated, symptoms progress and include growth retardation, diarrhea, impaired T-cell immunity, insufficient wound healing, infections, delayed testicular development in adolescence, and early death. Zn deficiency in adolescents is manifested by slow growth or weight loss, altered taste, delayed puberty, dwarfism, impaired dark adaptation, alopecia, emotional instability, and tremors. In severe cases, lymphopenia may occur; death follows an overwhelming infection. [28]

2.3.4 Toxicity

Zinc is relatively nontoxic. Nevertheless, high doses (1 g) or repetitive doses of 100 mg/day for several months may lead to disorders, especially gastrointestinal tract symptoms, decrease in heme synthesis due to an induced copper deficiency, and hyperglycemia. Exposure to ZnO fumes and dust may cause "zinc fume fever." The symptoms include chemically induced pneumonia, severe pulmonary inflammation, fever, hyperpnea, coughing, pains in legs and chest, and vomiting. [29]

2.3.5 Reference intervals for zinc

Zinc in serum: 70–120 μg/dL

Zinc in urine of normal subjects: 140–800 μg/24 hours

Zinc in urine of compliant patients on oral zinc therapy for Wilson's disease: >2,000 μg/24 hours

2.4 Previous study

A previous study was done by G. Murugan (2007) for evaluation of serum zinc levels on hemodialysis in this study, comparison of zinc status in patients with chronic kidney disease on hemodialysis with control group, reveals significant changes in the zinc metabolism. This is evidenced by statistically significant decrease in serum zinc levels in the study group suggesting zinc deficiency. Comparison of serum zinc status

between age and sex matched study and control group shows that there is no significant influence of age and sex on zinc levels. [30]

Another previous study was done by z. Ghoreshi, mosc, s.k. Ahaley, et al, (2011) in India for effect of hemodialysis on trace elements in patients with acute and chronic renal failure, and the result was as follow; Serum zinc levels were decreased quite significantly in acute as well as in chronic renal failure patients as compared to the control. The mean zinc levels were increased quite significantly after first hemodialysis as compared to pre-hemodialysis in both groups. [31]

Another previous study was done by Seyed Taher Esfahani, Mohammad Reza Hamidian, Abbas Madani, et al, (2006) for Serum zinc and copper levels in children with chronic renal failure, and the result was as follow; chronic hemodialysis may lead to changes in the serum levels of a number of trace elements, with the severity of the changes increasing with the duration of the hemodialysis. The deficiency of some trace elements especially Zn may contribute to various conditions and symptoms in children undergoing chronic hemodialysis. [32]

Chapter Three

Materials and Methods

3 Materials and Methods

3.1 Study design

Comparative analytical study, specifically it is case control study.

3.2 Study area

This study was conducted in Khartoum state, in Ribat University hospital.

3.3 Study period

This study was done during the period from March 2018 to June 2018.

3.4 Study population and sample size

One hundred blood samples were collected in this study.

Case group: 60 patients with chronic renal failure for different duration, age and including both males and females.

Control group: 40 apparently healthy individuals.

3.5 Study subject

Volunteers enrolled in this study were Sudanese patients with chronic renal failure, also apparently healthy individuals as control (age matched) were involved.

All volunteers was enrolled after being fully informed by the aim study, more over an informed consent was taken from every volunteer.

3.6 Sampling

Two and half ml of venous blood were collected by using sterile disposable syringes and poured into lithium heparin containers, immediately centrifuged and plasma was separated.

3.7 Selection criteria

3.7.1 Inclusion criteria

Test group: Sudanese patients with chronic renal failure.

Control group: Sudanese individual apparently healthy.

3.7.2 Exclusion criteria

Patients with chronic renal failure complaining from other disease like heart disease, diabetes mellitus and drugs containing zinc and copper were excluded.

3.8 Data collection

Data of this study was collected through interview using self-administered questionnaire.

3.9 Ethical consideration

Ethical approval was obtained from ethical committee of Shendi University and Informed consent will be taken from all the participants prior to their inclusion in the study. All the procedures will inform to the patients in their native language and informed written consent will be taken from them.

3.10 Ethical committee

Clearance from Shendi University ethical committee.

3.11 Quality Control

The precision and accuracy of all methods use in this study were checked at each batch using commercially prepared control sera.

3.12 Statistical analysis

Collected data were computed and analyzed by using the application of SPSS (statistical package for social sciences) version 21. The test used is a T test

3.13 Determination of serum copper and zinc

3.13.1 Instrument

The plasma level of zinc and copper determined by atomic absorption spectrophotometer (OPERATOR'S MANUAL January 2003 VER 3.94 C), and the obtained results were analyzed by SPSS.

3.13.2 Preparation of sample

For the determination of serum copper, dilute the sample with an equal volume of deionized water. For the determination of serum zinc, dilute the sample 1:5 with deionized water.

Samples are diluted with deionized water. The analysis is performed against standards prepared in glycerol to approximate the viscosity characteristics of the diluted samples.

3.13.3 Principle

The technique makes use of absorption spectroscopy to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law.

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law.

Chapter Four

Results

4.1 Results

This study was conducted in Khartoum state in Ribat University Hospital to evaluate the trace elements (Zinc and Copper) in renal failure patient.

This study includes 100 blood samples, a 60 from these sample were collected from patient with chronic renal failure and 40 samples were collected from health individual as control group. A total of 60 blood samples from patient with renal failure the males were 38 with 63% while the rests 22 were females with 37%, the age of population studied ranged between 17–90 years.

The result of the study was presented in tables and figures.

Table 4.1 Comparison between the means of plasma Zinc in control group and in case group

Study group	Number	Mean (mg/L)	Std.Deviation	P.value
Case	60	0.3	0.17	0.002
Control	40	0.7	0.14	

Table 4.2 Comparison between the means of plasma Copper in control group and in case group

Study group	Number	Mean (mg/L)	Std.Deviation	P.value
Case	60	0.5	0.30	0.019
Control	40	0.7	0.13	

Table 4.3 Comparison between the means of plasma Zinc and Copper in patient with age less than 40 years and in patient with age more than 40 years

Element trace	Age						P. value
	Less than 40 years			More than 40 years			
	No	Mean (mg/L)	Std.De	No	Mean (mg/L)	Std.De	
Copper	19	0.5	0.28	41	0.5	0.31	0.527
Zinc	19	0.2	0.16	41	0.3	0.18	0.434

Table 4.4 Comparison between the means of plasma Zinc and Copper in male and in female patient

Element trace	Gender						P. value
	Male			Female			
	No	Mean (mg/L)	Std.De	No	Mean (mg/L)	Std.De	
Copper	38	0.4	0.29	22	0.6	0.30	0.667
Zinc	38	0.3	0.18	22	0.3	0.16	0.578

Table 4.5 Comparison between the means of plasma Zinc and Copper in patient having a disease less than 6 years and patient having a disease more than 6 years

Element trace	Duration of disease						P. value
	Less than 6 years			More than 6 years			
	No	Mean (mg/L)	Std.De	No	Mean (mg/L)	Std.De	
Copper	41	0.5	0.30	19	0.4	0.28	0.621
Zinc	41	0.3	0.18	19	0.2	0.15	0.692

Chapter Five

Discussion
Conclusion
Recommendations

5.1 Discussion

The present study was carried out to investigate trace element (zinc and copper) among chronic renal failure patients in Ribat University Hospital, during the period from March to June 2018; 100 blood samples were collected, a 60 of these samples were collected from patient with renal failure as test group, and 40 samples were collected from health individual as control group.

The present study showed statically significant difference between the mean of the plasma levels of zinc and copper of the test group when compared with that of the control group, both are decreased. That illustrated in tables 4.1 and 4.2. This agree with (G. Murugan 2007) who was reported statically significant changes in the zinc metabolism. This was evidenced by statistically significant decrease in serum zinc levels in the study group suggesting zinc deficiency. Also similar study cared in India by (Z. Ghoreshi, S.K. Ahaley, et al, 2011) agree with serum zinc level, who report significant decreased in serum zinc level and disagree with serum copper level, who+ report no significant effect on serum copper level. [30, 31]

The results of this study showed insignificant difference between the plasma levels of zinc and copper of the test group according to the age. That illustrated in table 4.3. And also according to the gender. That illustrated in table 4.4. This agree with (G. Murugan 2007) who was reported statically no significant influence of age and sex on zinc levels when compare of serum zinc status between age and sex of study group.[30]

Also the results of this study showed that there was insignificant difference between the plasma levels of zinc and copper of the test group according to the duration of disease with. That illustrated in table 4.5.

5.2 Conclusion

This study concludes that:

- The plasma level of zinc in case is low than in control group.
- The plasma level of copper in case is low than in control group.
- The age of patients have no effect on the plasma level of zinc and copper.
- The gender of patients have no effect on the plasma level of zinc and copper.
- The duration of disease have no effect on the plasma level of zinc and copper.

5.3 Recommendations

This study recommends that:

- Other information should be collected about the patient like diet and its work.
- Further studies should be carried out with larger sample size and more trace elements such as iron.
- Suggest supplementation of zinc and copper may be helpful to improve the quality of life in patients undergoing dialysis.

Chapter Six

References
Appendices

6.1 References

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6.2 Appendix

Appendix (I)

Shendi University

Faculty of Graduate Studies and Scientific Research

Research questionnaire

Evaluation of plasma Zinc and Copper in Patients with Chronic Renal Failure in Khartoum State

The patient's questionnaire includes the following sections:

Section 0 – Questionnaire identification data (6) codes

Section 1 – Background characteristics (5) Questions

0 QUESTIONNAIRE IDENTIFICATION DATA

001 QUESTIONNAIRE IDENTIFICATION NUMBER |___|___|___|

002 CITY----------

003 HOSPITAL----------

004NAMES ----------

005 DATE OF INTERVIEW: __\ ____ \ _____

006 CHECKED BY SUPERVISOR: Signature _____ Date _____

Section 1: Background characteristics

No.	Questions and filters	Coding categories
Q101	Duration of disease	□less than 6 years □ more than 6 years □No Response
Q102	Age	□less than 40 years □ more than 40 years □No Response
Q103	Gender	□ …… □……
Q104	History of disease	□ Yes □ No

If no escape the following questions; but if yes, go to the following questions:

Q105	Other disease	□ Liver disease □GIT disease □Thyroid disease □Lipid disease □Other disease

If there is positive history of any of the above mentioned diseases we excluded this patient from our study.

Blood test result:

Copper Level: ……mg/L Zinc Level: ……mg/L

That is the end of our questionnaire. Thank you very much for taking time to answer these questions. We appreciate your help.

Appendix (II)